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(54) Title: CONDUCTING ELECTROACTIVE BIOMATERIALS

(57) Abstract

The development of conductive electroactive polymers which comprise a hydrophilic counterion provides biomaterials with properties which enable the incorporation and controlled release of biological macromolecules such as proteins. The improved properties of the polymers also enable the preparation of composite polymers which contains viable viruses or cells such as bacterial or animal cells. The composite polymers are also biocompatible and may be used as controlled drug delivery devices and biosensors, both *in vitro* and *in vivo*.

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TITLE: **CONDUCTING ELECTROACTIVE BIOMATERIALS**

TECHNICAL FIELD

The present invention relates to novel conductive electroactive biomaterials, and processes for making such biomaterials.

BACKGROUND ART

In the search for a new generation of biomaterials, it has been sought to mimic the cell membrane in designing new polymeric materials that not only have similar chemistry to the cell membrane but also are reactive. To date, the majority of biomaterials have been relatively inert materials whose properties are determined and fixed at the time of their synthesis. Furthermore, man-made biomaterials or at least their bio-interfaces tend to be homogeneous in composition. This contrasts with the structure of most interfaces within the body where the emphasis is not only on a heterogeneous blend of different molecules but the whole structure is dynamically active. This dynamic activity is maintained at the level of the cell membrane that has a resting potential that can be altered and that has a broadly hydrophobic lipid component in which a mosaic of protein and carbohydrate structures are dispersed. The compositi n can be changed in time and some components, mucopolysaccharides and proteins, can be secreted from it in response to specific stimuli and as such current bi materials are far from suitable.

Conductive electroactive polymers, such as polypyrrole, which have been previously described have a range of properties that would appear to be useful in the design of new biomaterials. They are dynamically active, have a resting potential and can be synthesised to contain significant amounts of protein which remains bioactive. Application of small electrical potentials can alter the activity of enzymes incorporated into such polymers or of the binding of antibody to antigen. However, this protein cannot be readily released from polypyrrole : protein polymers upon reduction, thus limiting their application. There is thus a need for a polymer which preferably can release biological macromolecules and other biological materials incorporated therein and which, in preferred embodiments, is biocompatible and can be used for applications as both a biomaterial and a controlled drug delivery device.

Accordingly, it is an object of the present invention to provide means for avoiding or at least ameliorating some of the above discussed disadvantages of the prior art.

DISCLOSURE OF INVENTION

As used throughout the specification and claims the term "biological macromolecule" refers in the context of the present invention to any molecule of biological origin which has a high molecular weight and may be, but not necessarily, of polymeric construction. Examples of such macromolecules are proteins, lipids, carbohydrates, nucleic acids and the like. As used in the present invention the term "macromolecular complex" refers to larger macromolecular structures of increased complexity and includes viruses, bacteria, fungi and plant and animal cells. The term "hydrophilic counterion" refers in the context of the present invention to a counterion which when incorporated into the composite polymer of the invention causes the composite polymer to have a high water content. By the term "polyelectrolyte" is meant a counterion which is a multi-charged species. The term "cytopolymer" refers to a composite polymer which incorporates biological materials such as viruses, bacteria, fungi or plant and animal cells. Throughout this specification by "macroscopically uneven" is meant that the distribution of polymer was such that there were bare patches of gold not covered by polymer. By "microscopically uneven" is meant that red blood cells (RBC) were distributed so that there were microscopic fields at 40x magnification that did not contain red blood cells.

In one aspect the invention consists in a conductive electroactive composite polymer comprising a hydrophilic counterion in combination with a biological macromolecule and/or a biological macromolecular complex.

In another aspect the invention consists in a process for making a conductive electroactive composite polymer having a hydrophilic counterion, comprising the step of oxidising a monomer in the presence of a hydrophilic counterion and a biological macromolecule and/or a biological macromolecular complex.

In a preferred embodiment, the monomer of choice for the preparation of the composite polymers of the invention is pyrrole. However, other monomers or mixtures of monomers may be used, provided that they are compatible with the biological macromolecule or the biological macromolecular complex. Thus, aniline could also be a suitable monomer when the process used for its polymerisation satisfies the compatibility criterion. Similarly, thiophene may also be used in the preparation of the polymer if a suitably water-soluble derivative of thiophene is used. Preferably the polymerisation process is carried out by electro-oxidation but suitable biochemical or chemical means of oxidation or reduction could also be used.

In another preferred embodiment, the hydrophilic counterion is a polyelectrolyte selected from the group consisting of polyvinyl sulphonate (PVS), dextran sulphate, chondroitin sulphate, polyglutamic acid, polyacrylic acid, heparin sulphate, hyaluronic acid and mucopolysaccharides. Typically, the concentration of the counterion is about 250 to 500 mg/100 mL. However, as will be appreciated by those skilled in the art any suitable counterion can be used in the present invention.

In yet another preferred embodiment, the incorporated biological macromolecule is a protein, a glycoprotein or a lipoprotein, such as an enzyme, a hormone, a growth factor, a cytokine or an integral cell membrane component such as a receptor or a specific cell surface. However, other proteins or mixtures of proteins may be used. In a highly preferred embodiment the biological macromolecule is releasable from the composite polymer.

In another highly preferred embodiment the invention consists in a composite conductive, electroactive polymer in communication with a virus or a bioactive cell which

can be of bacterial, fungal, plant or animal origin. This type of composite polymer will be referred to as a "cytopolymer".

In yet another highly preferred embodiment, the polymer of the invention comprises a polymeric composite of polypyrrole:PVS in communication with a red blood cell.

In another aspect the invention consists in a method of delivering a biological macromolecule contained within a conductive electroactive composite polymer into an environment surrounding the polymer, said polymer comprising a hydrophilic counterion in combination with a biological macromolecule and/or a biological macromolecular complex, said method comprising the step of:

applying a stimulus to said composite polymer to release said macromolecule into said surrounding environment from said composite polymer.

In yet another aspect the invention consists in a method of detecting a ligand comprising the steps of:

a) introducing a conductive electroactive composite polymer comprising a hydrophilic counterion in combination with a biological macromolecule and/or a biological macromolecular complex into an environment containing a ligand which specifically binds to said macromolecule and/or macromolecular complex contained within the composite polymer;

b) allowing the ligand to bind to said macromolecule and/or macromolecular complex;

c) measuring the change in electrical properties of said composite polymer and thereby determining the presence and/or concentration of the ligand.

In a further aspect the invention consists in a method of preventing or treating a disorder caused by or associated with a deficiency or absence of a biological macromolecule and/or a biological macromolecular complex comprising the step of administering to a host requiring such treatment a conductive electroactive polymer described above.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The polymer made in accordance with the present invention has been found to have interesting hydrogel like properties of high water content and biocompatibility as

well as the capacity for controlled release of bioactive protein and maintenance of cell integrity and viability. The biocompatible polymer is also electroconductive and electroactive. The electroactivity provides a mechanism for the control of several key properties for example the release of molecules such as drugs, proteins and other macromolecules and, if whole cells are incorporated, enables controllable modification of the cell membrane and cell activity.

The improved mechanical properties of the biocompatible polymers of the present invention over hydrogels and the facile control of their properties by application of small electrical potentials make them interesting candidates for the design and synthesis of a new generation of smarter biomaterials. Furthermore, the release of proteins from polymers is of interest for applications as both biomaterials and controlled drug delivery devices.

The novel properties of the biocompatible polymers enable the incorporation of very complex macromolecular structures such as viruses and even whole cells of bacterial, plant or animal origin. The viable cells can be incorporated directly during the polymerisation process with minimal lysis of the cells, thus producing a composite polymer comprising intact cells which remain biologically active.

Such cytopolymers may be useful eg. as cell carriers or as diagnostic tools for the detection of antibodies or analytes via ligand-cell binding or interaction. The analyte-cytopolymer interaction may also be translated into a range of electrical signals since the composite polymer is electroactive and conductive, thus rendering the cytopolymers potentially useful as novel biosensors. *In vitro* study or characterisation of cell-ligand interaction may also be conducted using such structures, as well as modulation of cell function and/or structure.

The cytopolymer may be used as a biosensor for determining eg. blood type or group as described below. In this regard, as far as making a sensor responsive to a particular blood group, it is necessary that the sensor contain an appropriate type of cell.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 represents a view f the electrochemical cell used for polymer growth.

Figure 2 represents a chronopotentiogram of polymer growth of (A) polypyrrole: PVS: horseradish peroxidase; (B) polypyrrole:PVS:RBC (monomer solution = 0.1M

pyrrole, 1 g/L PVS and red blood cells, in 92.4 g/L sucrose solution); (C) polypyrrole:heparin sulphate:RBC (monomer solution = 0.1 M pyrrole, 2.5 g/L heparin sulphate and red blood cells in 92.4 g/L sucrose solution), (D) polypyrrole:dextran sulphate (monomer solution = 0.2 M pyrrole, 5g/L dextran sulphate 50,000 in water), and (E) polypyrrole:dextran sulphate:protein (monomer solution = 4 mL/L of albumin, 0.2 M pyrrole and 5 g/L dextran sulphate 50,000 in 92.4 g/L sucrose solution). Growth conditions = current density of 0.5mA/cm^2 for 0.5 min. in case of (A) to (C), or 1.0 mA/cm^2 for 1.0 min. in case of (D) and (E); area of electrode = 20 cm^2.

Figure 3 represents a cyclic voltammogram of (A) polypyrrole: PVS: horseradish peroxidase in 1.0 M NaCl, (B) polypyrrole:PVS:RBC (monomer solution = 0.1M pyrrole, 1 g/L PVS and red blood cells, in 0.27M sucrose solution, (C) polypyrrole:heparin sulphate:RBC (monomer solution = 0.1 M pyrrole, 2.5 g/L heparin sulphate and red blood cells in 92.4 g/L sucrose solution), (D) polypyrrole:dextran sulphate (monomer solution = 0.2 M pyrrole and 5g/L dextran sulphate 50,000 in water), and (E) polypyrrole:dextran sulphate:protein (monomer solution = 4 mL/L of albumin, 0.2 M pyrrole and 5 g/L dextran sulphate 50,000 in 92.4 g/L sucrose solution). Growth conditions = 0.75mA/cm^2 for 1 min. for (B) and (C) and 1.0mA/cm^2 for 1.0 min. for (D) and (E). Cyclic voltammetry was conducted at a scan rate of 40mV/sec. in either 1.0M NaCl, for (A) and (D), or 0.15M NaCl, for (B) (C) and (E).

Figure 4 represents an electrochemical quartz crystal microbalance (EQCM) study of (A) polypyrrole:chondroitin sulphate and (B) polypyrrole:PVS, cycled between 0.2V and -0.8V in 1M NaCl (scan rate = 10 mV/sec; area of EQCM electrode = 0.25cm^2)

Figure 5 represents an atomic force micrograph of polypyrrole polymer containing red blood cells. Polymer was fixed in Zamboni solution, (growth conditions = galvanostatic at current density of 5.0mA/cm^2 for 1 min.)

Figure 6 represents a cyclic resistometry study of (A) polypyrrole: chloride (1) and chondroitin sulphate (2), and (B) polypyrrole:dextran sulphate (scan rate = 10mV/sec).

Figure 7 represents a EQCM trace of polypyrrole:chondroitin sulphate during dehydration and rehydration.

Figure 8 represents a study of (A) protein incorporation into the polymer during synthesis, as a function of the amount of protein in the synthesis solution (current density = 1mA/cm^2), and (B) protein release from the polymer on stimulation (fixed potential of -0.9 volts). (□ = growth at pH 9.6; ● = growth at pH 7.0).

Figure 9 represents a cell culture of PC12 cells on polypyrrole:chondroitin sulphate in the presence of soluble nerve growth factor (NGF).

Figure 10 represents a high resolution light micrograph of red blood cells incorporated into a polymer.

Figure 11 represents a scanning electron micrograph of a polymer incorporating red blood cells.

Figure 12 represents microscopic appearance of red blood cells in (A) monomer mix containing 0.2M pyrrole and 1g/L PVS in isotonic sucrose, and (B) isotonic sucrose solution alone.

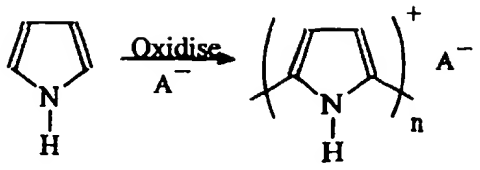
Figure 13 represents mass changes during cyclic voltammetry of (A and B) a thin polypyrrole:PVS:RBC polymer (growth conditions = galvanostatic at current density of 0.5 mA/cm^2 for 1.0 min.), and (C and D) a thick polypyrrole:PVS:RBC polymer (growth conditions = galvanostatic at current density of 5.0 mA/cm^2 for 1.0 min.). The polymers were cycled in 0.15M $NaNO_3$, in the absence (A and C) or presence (B and D) of 40µl of anti-Rh(D) monoclonal antibody. Scan rate = 50mV/sec.

Figure 14 represents cyclic resistometry study of polypyrrole:PVS:RBC polymer in the presence of (A) Rh control serum and (B) anti-D antibody (growth conditions = 0.5 mA/cm^2 for 1 min. in 0.15M $NaNO_3$, scan rate = 25mV/sec.)

EXAMPLES

Example 1: **Preparation of Composite Supramolecular Assemblages**

A positively charged homopolymeric backbone, which is counterbalanced by polyanionic complex carbohydrates such as dextran sulphate or chondroitin sulphate can be synthesised according to:



wherein A^- is a hydrophilic polyelectrolyte counterion incorporated during polymerisation. The presence of the counterion provides a facile route for the incorporation of chemical and biochemical functionalities eg. biologically active proteins, enzymes, cells.

Composite supramolecular assemblages were synthesised by electrooxidation of pyrrole monomer in the presence of proteins and polysaccharides in their anionic forms. Intrusion of other anions was limited by extensive dialysis of the macroanions against water (purified to 18 mega Ω) or use of salt free reagents, for example isotonic sucrose in the case of cells. To facilitate future cell culturing experiments composites were synthesised on transparent foils of mylar coated with gold (Cortaulds). Composites were grown by applying a constant electrical current (galvanostatic growth) at a density of $0.5mA/cm^2$ for 0.5 to 4 min. The electrochemical cell was designed to provide a uniform parallel electrical field over the entire electrode and to eliminate or minimise edge effects. Referring to Figure 1, the electrochemical cell comprises a cell body 1 which contains the first electrode 2 made of reticulated vitreous carbon, the second electrode 3 coated with gold foil and a third electrode 4 which is a reference electrode (Ag/AgCl) comprising a salt bridge 5 (0.15M NaCl). The reference electrode is included to measure the potential.

The solution in the electrochemical cell contained pyrrole distilled and purified over alumina by standard techniques immediately before use. In this regard pyrrole as supplied from Merck was distilled in standard equipment and the fraction distilling between 130-131°C was collected and stored in the dark under nitrogen in a deep freeze (approximately -10°C). Immediately before use the pyrrole was passed over alumina to remove any coloured contaminants. The concentration of pyrrole may be varied, preferrably between 0.1M and 0.3M. The optimal concentration could be determined by those skilled in the art with the aid of the present disclosure. The following polyanions and complex carbohydrates were used as counterions: polyvinyl sulphonate (PVS, M_r

900-1,000 Aldrich), Dextran sulphate (M_r 50,000 Sigma or 1,000,000 Fluka), chondroitin sulphate (Sigma), Heparin sulphate (Sigma, Grade 1-A from porcine intestinal mucosa, H3393), polyglutamic acid (WAKO Chemicals), polyacrylic acid (M_r 400,000, Polysciences), at concentration of from about 0.5 to 5.0 g/L. Chronopotentiograms recorded during growth indicated that a conducting polymer material was formed (Figure 2).

Depending on the chemical composition of the polymer, the prepared polymer films may be stored for periods of up to 9 to 12 months at room temperature, without deleterious effects on the properties of the polymer.

Characterisation

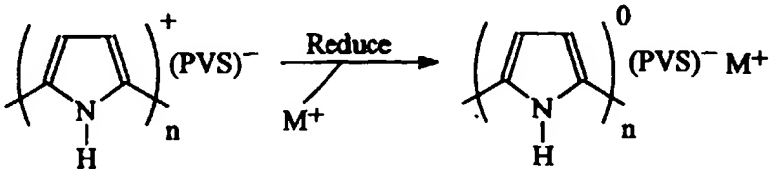
Cyclic voltammetry, cyclic resistometry and electrochemical quartz crystal microbalance (EQCM) studies were performed as previously described (see John R, Wallace, G G, J. Electroanal. Chem. 1993, 354, 154-160; John R, Talaie A, Fletcher S and Wallace G G, J. Electroanal. Chem. 1991, 319, 365-371, and Mirmocheni A, Price W E and Wallace G G, J Membrane Science, 1995, 100, 239). Cyclic voltammetry was performed using a BAS CV27 potentiostat, a three-electrode cell in an electrolyte of 0.15M or 1.0M NaCl. Scan rates of 25 - 50 mV/sec were employed. For EQCM crystals were cleaned in 0.4M H_2SO_4 for 10 min. and then in 20% potassium ferrocyanide for 10 min. Polymers were grown galvanostatically as described above then washed in 0.15M NaCl or 0.15M $NaNO_3$. For resistometry gold electrodes of 1 cm^2 were used. Synthesis and cyclic voltammetry were performed as described in an electrolyte of 0.15M or 1.0M NaCl. Water content was estimated by gravimetric analysis after either drying over phosphorus pentoxide or on a vacuum pump overnight. Dynamic changes in water content were determined by using a quartz crystal microbalance. Enzyme activity of horseradish peroxidase (HRP) was determined using 2,2'-Azinobis(3-ethylbenzthiazoline sulphonic acid, (ABTS) as a substrate (see Gallati Von H, J. Clin. Chem. Clin. Biochem. 1979 17, 1 and Porstmann B, Portstmann T and Nugel E, J. Clin. Chem. Clin. Biochem. 1981 19, 435).

Rat PC12 phaeochromocytoma cells were cultured in Dulbecco's Modification of Eagles Medium (DMEM) supplemented with foetal bovine serum (10% v/v) and with

heat inactivated horse serum (5% v/v). No antibiotics were used and the cultures were maintained at 36.5°C in an atmosphere of CO_2 (5% v/v). The polymer films tested were cut to a size that fitted into the bottom of rectangular multi-well culture dishes (Nunc cat. no. 176600). Cells were seeded at 10^4 cells/cm^2 and left for 2 to 4 days before the polymer was cycled through potentials -0.8V to +0.7V (vs Ag/AgCl) using ElectroLab software. Cells were observed by phase contrast microscopy or were fixed with formaldehyde (4% w/v) and stained with haematoxylin and eosin or by silver impregnation (see Mallory F B, Pathological Technique 1961 pp 158-180 Hafner, New York). Nerve growth factor was added to the culture medium at a concentration of 8ng/mL and neurite growth monitored after 48 to 72 hours.

Results

All polymers grew at potentials between 1.3V and 0.6V, with the potential becoming steadily less anodic throughout the polymerisation period, indicating growth of a conducting polymer. This was true even for transparent polymers. The thickness of the film could only be estimated at edges or at faults in the polymer using atomic force microscopy. It was found that films grown for 30 sec. from monomer solutions that did not contain red blood cells, were approximately 100nm thick and when grown for 4 min they were 700-1000 nm thick. Cyclic voltammetry in 1M NaCl showed a picture typical of polypyrrole with an immobile anion as dopant (Figure 3). Compared to polypyrrole chloride, the peak of the oxidative wave was shifted to negative potentials -0.2 to - 0.4 V Ag/Ag Cl. This peak has been associated with cation movement in/out of the polymer according to:



Quartz crystal microbalance studies showed that reduction was accompanied by mass increase (see Figure 4) confirming this scenario for most of the polymers. Interestingly mass increases were observed even at relatively anodic potentials (i.e. 0.20V

to 0.00V). Polymers containing PVS, especially for polymers grown for longer than 4 minutes, were a special case where a biphasic response was observed.

Scanning electron microscopy showed that the polymers were relatively amorphous when thin. This was confirmed by atomic force microscopy (AFM) which was also used to confirm the presence of red blood cells in the polymer (Figure 5). The root mean square roughness of the polymer was 13.8 nm over a 5 X 5 μm area. The average peak to valley distance at faults in the polymer (eg. edges) was 92 nm. This gives a measure of total thickness.

Cyclic resistometry showed that for all polymer systems the polymer resistance increased on reduction but the polymers remained relatively conductive when reduced (see Figure 6) compared to polypyrrole chloride that shows a resistance change of 100-200 Ω when reduced. The polymers were also less conductive than polypyrrole chloride when in the fully oxidised state (60-70 Ω vs 40-50 Ω).

Gravimetry of the polymers after drying showed a high content of water. For thin polymers (30 - 60 sec. growth) 80% of the weight immediately after synthesis was water. This depended on the atmospheric relative humidity on the day of synthesis (see Table 1 below).

TABLE 1: Percentage water content of different polymers at different atmospheric relative humidities.

	Relative Humidity		
	43%	74%	80%
Polymer			
PP/PVS	39%	53%	60%
PP/Dextran. Mr 1,000,000	42%	57%	63%
PP/Dextran. Mr 50,000	38%	52%	67%

Quartz crystal microbalance studies showed that this water was rapidly removed by desiccant and was reabsorbed when the polymer was exposed to a water saturated atmosphere (see Figure 7).

Example 2: Dynamic polymer studies: Inc rporation and release of proteins

To evoke release of species from the polymer, potential step and potential cycling experiments were carried out in a three-electrode cell under potential control from an EGG 173 potentiostat with MacLab data recording or ElectroLab potential control. Release was carried out into acetate buffer pH 5.0 or phosphate buffer pH 8.5 for time periods ranging from 1 to 20 minutes. A variety of commonly used buffers may also be suitable for this purpose.

Horseradish Peroxidase

Enzyme assay showed that appreciable HRP activity could be incorporated into the polymer during synthesis of PVS:HRP:pyrrole composite. Longer synthesis times showed lower activity measured in the polymer.

Cycling the potential from +0.6 to -0.5V caused release of large amounts of enzyme (2600mU). To determine whether this was released during oxidation or reduction, potential steps were carried out. Pulsing the potential from rest (approx. 0.00V vs Ag/AgCl) to negative potentials (-0.5V vs Ag/AgCl) caused release of enzyme optimal after 5 mins (1674mU) (see Table 2 below). Soaking the polymer released measurable but low release (215mU) and pulsing to positive potentials caused intermediate release (556mU).

TABLE 2: HRP activity released from composite polymers upon application of potential.

	Activity in Polymer (mU)	Total Activity Released (mU)	Activity Remaining (mU)
Control	5580	214.7	ND
5min at -0.5V	5580	1674.0*	271
10min at -0.5V	5580	494.8	134
5min at +0.6V	5580	556.0	ND

Legend: Control polymers were soaked in acetate buffer for 5 minutes. Constant electrical potential (-0.5V or +0.6V) was applied for 5 or 10 minutes to stimulate polymers in 13 mL of acetate buffer. ND = not determined; * $p<0.1$; $n=10$.

Haemoglobin

Using haemoglobin (Mr 64,500, pI 6.8, a neutral protein) as a test macromolecule it could be demonstrated that the concentration of pyrrole not only affects incorporation of protein into the polymer but also affects release.

Table 3 shows that as the pyrrole concentration is increased from 0.1M to 0.5M the extent of incorporation of protein is reduced. Furthermore, the amount of protein released from the polymer is significantly reduced (Table 4). This effect is probably caused by the denaturing effect of the hydrophobic solvent pyrrole.

TABLE 3: Effect of pyrrole concentration on the incorporation of haemoglobin into polypyrrole polymers.

Current Density (mA/cm^2)	Growth Time (minutes)	Coulombs	Percent Incorporation*	
			0.1M pyrrole	0.5M pyrrole
0.5	0.5	0.25	0.317	0.26
1	0.5	0.5	0.431	0.329
0.5	1	0.5	0.752	0.377
1	1	1	0.55	0.393
0.5	2	1	0.778	0.294
1	2	2	0.74	0.302
0.5	5	2.5	0.799	0.257
1	5	5	0.945	0.384

* total amount of protein incorporated into the polymer, expressed as a percentage of the total amount of protein present in the monomer solution at the time of polymerisation.

TABLE 4: Effect of pyrrole concentration on the release of haemoglobin from the polypyrrole polymers.

Current Density (mA/cm^2)	Growth Time (minutes)	Coulombs	Percent Release*	
			0.1M pyrrole	0.5M pyrrole
0.5	0.5	0.25	16.18	45.73
1	0.5	0.5	57.13	18.66
0.5	1	0.5	29.25	1.94
1	1	1	63.59	26.34
0.5	2	1	57.36	47.69
1	2	2	52.61	7.79
0.5	5	2.5	50.02	35.63
1	5	5	59.21	39.61

* total amount of protein released from the polymer with the application of -0.8V for 2 min., expressed as a percentage of the total amount of protein incorporated into the polymer.

The extent of protein incorporation was investigated as a function of growth time and current density. Such studies show that as growth time is extended the amount of protein incorporated is increased. At lower current densities the amount of protein is almost linearly related to growth time but at higher current densities a levelling off occurs as growth time is extended.

Human Serum Albumin

Experiments performed with human serum albumin (HSA, Mr 70,000, pI 5.5, an anionic protein) demonstrated that polymers contained an amount of protein that was a function of the amount of protein in the synthesis solution. Between 1 to 2% of the protein within the monomer solution was incorporated into the polymer.

The HSA was retained within the polymer and did not diffuse from it when incubated in saline solution, 0.15M NaCl; after 7 hours 98 (±3)% remained and even after 3 days 95 (±10)% remained. When the polymer was reduced by applying a cyclic voltage ramp from +0.5 V to -0.7, -0.8 or -0.9 V, appreciable protein was released into the saline electrolyte solution. The amount of protein released was greater for more negative potentials. The time course of release was investigated by applying a fixed negative potential for times ranging from 2 to 32 seconds. The protein was released rapidly from the polymer. Within 2 seconds 20 to 30 percent of the protein contained within the polymer had been released. After only 32 seconds most of the contents of the polymer had been released. When oxidising potentials (+0.5 V) were applied or potentials close to the rest potential of polypyrrole (+0.17 V) no protein was released.

Nerve Growth Factor

Using mouse salivary nerve growth factor (NGF, Mr 26,000 pI 9.6 a cationic protein), it can be demonstrated that during synthesis the amount of protein incorporated into the polymer was linearly related to the amount of protein in the synthesis solution (Figure 8 A). Depending on the pH of the synthesis solution adjusted with either NaOH or HCl, between 1.4 and 2.0% of NGF in the solution was taken up into the polymer.

Incorporation of NGF was greater at pH 9.6, the isoelectric point of NGF, consistent with the protein being incorporated by physical entrapment in the polymer.

At pH 7.0 NGF is positively charged and when mixed with an anionic polyelectrolyte such as dextran sulphate will form ionic complexes. Since there is a vast excess of dextran sulphate which is highly negatively charged (approximately 1,000 negatively charged sulphates per molecule, Mr=50,000) the NGF will only minimally diminish the net negative charge. It could however change conformation and will increase the molecular mass significantly by 50% (26,000) for each NGF molecule bound. This could be the reason for the decreased levels of incorporation at the lower pH.

As for HSA above, release was induced by application of negative potentials (see Figure 8B). Applying -0.7V produced some release of NGF. However, release evoked at pH 7.0 was detected only 64 seconds after the application of potential. When more negative potentials were applied (-0.9V) greater and more rapid release was detected within 2 seconds of application of potential. The amount of protein released increases with the time of application of potential so that up to 45% of the NGF contained in the polymer was released within 1 minute. Soaking a polymer containing NGF in 0.15M NaCl at 4°C for 3 - 4 weeks caused no more growth factor to diffuse out of the polymer.

Importantly, the mode of release of NGF is such that no protein leaks out of the polymer by diffusion but within 2 seconds of application of the electrical potential significant protein is released. Such control of protein release from a polymer has not been achieved before. The amounts of NGF released are sufficient to cause differentiation of PC12 cells. The PC12 cells will begin to differentiate when NGF concentration in culture medium reaches about 16ng/mL. The maximum NGF released in the above study was 450ng in 1 minute. Release of this amount of NGF into a 4 mL culture volume would be more than sufficient to cause stimulation of PC12 cells growing upon the polymer.

Factors that affected the extent of protein incorporation were the time of synthesis and the concentration of pyrrole in the monomer solution. The mechanism of release of the protein is thought to be expulsion of ani ns from the polymer when it is reduced. The open, porous, hydrophilic matrix is responsibl for the facile expulsion of the negatively charged protein. Even though the protein is expelled as an anion it must be retained

within the polymer by more than ion exchange. Simple anions like chloride diffuse from the polymer by ion exchange. There was no such tendency for albumin to diffuse despite the high water content. Therefore, there must be other forces retaining the protein within the polymer matrix. This could include hydrophobic effects as well as ion pairing, hydrogen bonding and steric restriction.

Example 3: Biocompatibility

In vitro biocompatibility of the polypyrrole composite polymers was investigated by their ability to support growth of a variety of cells including breast cells, fibroblasts and in particular rat PC12 cells. The latter are particularly fastidious and the ability to support NGF-induced differentiation is a stringent indicator of biocompatibility. It was found that rat PC12 cells adhered to and multiplied on all polypyrrole surfaces tested. Some of the new composites synthesised in this study proved inferior to those reported previously. Polypyrrole polyglutamic acid was a poor substrate. Cells adhered poorly when counted at 4 hours after plating. The percentage of cells adherent to different polyglutamate polymers ranged from 19 to 45% (mean = 25.6%) whereas identical cells plated on polypyrrole : dextran sulphate were 98% adherent and on polystyrene coated with poly L-lysine were 78% adherent. PC12 cells plated on polypyrrole : polyglutamate preferred to adhere to occasional patches of bare gold that had not been coated by polymer. Nevertheless, cells once adherent on all polymers tested were still able to differentiate in response to exogenous nerve growth factor (8ng/mL) added to the culture medium (see Figure 9). As can be seen from Table 5 below, the composites made in accordance with the present invention provide very low water contact angles which is a result of the hydrophilic nature of the polymer system.

TABLE 5 Hydrophilicity of composite polymers.

	Hydrophilicity (Contact Angles - H_2O)
PP/PVS	22.2 ± 3.2
PP/DEX	8.1 ± 2.4
PP/dex	14.5 ± 5.0
PP/CS	26.1 ± 2.0
Gold Film	73.1 ± 3.6

The ability of cells to adhere and grow on the polymers depended on the nature of the polymeric counterion. Two parameters could influence this, the charge density on the backbone and its hydrophilicity. Polymers containing polyglutamic acid were the worst substrate of those tested. All had a slightly different density of negative charge. PVS had the highest charge density and dextran and chondroitin sulphates the lowest, polyglutamic acid was intermediate. The charge density can have profound effects on the polymer. At high charge density it is likely that not all of the charges would be paired with positive charges on the pyrrole backbone. Excess negative charges would be paired with cations from the solution and would contribute to the high water content of the polymers. Glutamic acid is relatively hydrophobic when protonated. Thus it is thought that those groups strongly ion paired with positive charges on the backbone would contribute a hydrophobic nature to the composite compared to the hydrophilic carbohydrate units.

The facile synthesis of polymers containing extracellular complex carbohydrates such as chondroitin sulphate means that a new class of materials that have better mechanical properties than hydrogels yet contain functionalities that are components of natural biointerfaces is possible. The fact that the composite is electroactive means that the material is capable of dynamic control of key properties.

Example 4: Cytopolymers

Human red blood cells collected at the Red Cross Blood Bank from anonymous donors were fractionated from whole blood and were stored in Citrate, Adenine, Phosphate, Dextrose (CAPD) solution. Before use they were washed and suspended in isotonic media such as Adenine, Citrate, Dextrose (ACD), CAPD, 0.9% w/w saline or 92.4 g/L sucrose. A monomer solution was then made from the packed cells. A typical monomer solution contained pyrrole at concentrations from 0.1 to 0.3M, an osmotic agent and/or an energy source, such as sucrose at a concentration of 92.4 g/L, a polyelectrolyte selected from polyvinyl sulphonate (PVS), dextran sulphate, chondroitin sulphate, polyglutamic acid, polyacrylic acid, heparin sulphate, hyaluronic acid or mucopolysaccharides, for example polyvinyl sulphonate at a concentration of 1 g/L and red blood cells at 3 to 6 x 10^{12}/L.

Integrity of red cells in the monomer mix before and after synthesis of polymer was performed by determination of a full blood count on a Coulter S-Plus IV, electronic

cell counter. The state of the haemoglobin in the monomer mix was determined by analysis on a Radiometer blood gas analyser. The integrity of AB and the D-antigen on the cells was determined by performing a ABO and Rhesus blood group typing using standard reagents. Morphology of red blood cells in the monomer mix was determined by May Grunewald Geimsa staining.

Polypyrrole:PVS:RBC Cytopolymer

Polymers were grown on plastic foil coated with gold in a standard electrochemical cell (see Figure 1) using galvanostatic growth at 0.5-1.5 mA/cm^2 for 0.5 to 1.5 minutes, temperature was maintained at less than 17°C. Higher or lower temperatures can be used but, as is well known, synthesis of any type of polypyrrole polymer is temperature sensitive; lower temperatures usually producing polymers with greater electrical-conductivity and better mechanical properties. Lower temperatures are also desirable because it enables the production of polymers that are macroscopically even and preserves the integrity of biological components.

Red blood cells that were incorporated into the polymer appeared intact at high resolution light microscopy but not always in their usual biconcave form (Figure 10) This may be an artefact of fixation caused by the picric acid in the fixative used. Other fixatives can be used but picric acid present as a modified Zamboni fixative composed of 4% formaldehyde, 0.25% glutaraldehyde, 40% v/v saturated picric acid, in 0.1M sodium phosphate buffer pH 7.4 gave superior results. Formaldehyde vapour fixation better preserves the biconcave form but is not suitable for large-scale use. The biconcave form or morphology of the red blood cell is not important in itself. It is the state of antigens/enzymes etc. in the membrane as a function of the use of the cytopolymer which is crucial. Scanning electron microscopy shows that the red blood cells are incorporated into as well as onto the polymer (Figure 11).

Effect of pyrrole solution on red blood cell integrity

Pyrrole concentrations at 0.5M caused complete (100%) lysis of red blood cells and at 0.1-0.3M haemolysis of red blood cells in the monomer mix was between 1-5% and depended on the age of the red blood cells. Extended exposure of red cells to 0.3 M pyrrole increased the haemolysis and caused denaturation of AB and Rh(D) antigens.

Therefore, monomer solutions were prepared fresh and used within 60 minutes of preparation. The preferred method involves cooling the monomer mixture to less than 17°C, performing electropolymerisation at this low temperature and using pyrrole concentrations below 0.15M.

Histology of red cells in the monomer mix showed reasonably good morphology (Figure 12). There were some burr cells and occasional acanthocytes. Electronic counting showed that mean cell volume, mean cell haemoglobin and cell counts (in the monomer mix) were within normal limits, both before and after synthesis of polymer. The normal limits in the laboratory in which the experiments were conducted were:

MALE: Mean cell volume: 80.8 - 97.7fL,
Mean cell haemoglobin: 27.0 - 32.2 pg,
Cell: 4.6 - 5.81 x 10^{12}/L

FEMALE: Mean cell volume: 80.6 - 98.4fL,
Mean cell haemoglobin: 26.8 - 32.5 pg,
Cell: 3.91 - 5.23 x 10^{12}/L.

More "even" polymers can be grown by reducing the concentration of red blood cells to 1.5 to 3.0 x 10^{12}/L.

It was found that inclusion of CAPD and ACD caused the polymer to be macroscopically and microscopically uneven. Cells washed in sucrose produced more even polymers.

The conditions for and the stability of the cytopolymers on storage is to a larger degree governed by the type and viability characteristics of the cells incorporated in the polymer and to a smaller extent by the chemical composition of the polymer.

Signal Generation: Electrochemical Characterisation of the Polypyrrole:PVS:RBC Cytopolymer

Cyclic Voltammetry/EQCM:

A polypyrrole:PVS:RBC:sucrose polymer was cycled in 0.15M $NaNO_3$ in the presence of anti-D antibody (the antibody was monoclonal, at a concentration of 1mg/mL, obtained from Gamma Biologicals, USA). A polypyrrole:PVS polymer which does not

contain red blood cells served as a blank polymer, where no interaction involving red blood cells could take place.

Firstly, thin polymers (i.e. grown at 0.5 mA/cm^2 for 1 min.) showed markedly different ion fluxes to thick ones (grown at 5.0 mA/cm^2 for 1 min.). Thin polymers showed a mass increase on oxidation whereas thick polymers showed a mass increase on reduction as well as on oxidation. This indicates that anion exchange is predominant in thin polymers whereas both cation and anion exchange processes occur on thicker polymers.

Upon addition of anti-D subtle changes occurred in cyclic voltammograms. Significant changes were observed in the mass-potential profiles obtained for thinner polymers. The changes observed indicate that the anion exchange capacity of the cytopolymer has increased upon addition of anti-D. Upon addition of anti-D to thicker polymers it appears that anion capacity is again influenced, but now it decreases. No interaction was seen on a blank polymer that did not contain red blood cells.

Cyclic voltammetry showed that a good electroactive polymer film was produced. There was no interference due to the iron present in the haemoglobin. The cyclic voltammogram was similar to polymer grown with only PVS as a counterion.

Studies into Signal Generation were carried out using electrochemical EQCM experiments to quantitate the ion fluxes and mass changes that caused the qualitative changes.

Mass measurements during application of ramped potentials identical to those used for cyclic voltammetry showed profound changes when anti-D was added (Figure 13).

The net mass of the polymer increased as would be expected when antibody was bound to the polymer. This was not artificial as shown by the absence of mass increase when the antibody was added to a blank polymer that did not contain red blood cells.

Furthermore, ion fluxes measured during potential cycling showed striking irreversible changes. These changes are presumably due to hitherto unseen ion fluxes caused by applying potentials to polymers containing intact red cells. These fluxes could easily be modified by binding of antibody to the D-antigen.

These experiments therefore show that interaction of Rh^+ blood containing polymers with anti-D immunoglobulin produces a variety of electrochemical changes. These changes or flux provide the basis for the generation of a robust electrical signal useful for blood grouping according to the antigen type present in the cytopolymer.

Cyclic Resistometry:

This technique measures the resistance while the potential is ramped between two limits. An electroactive polymer shows a characteristic sigmoidal resistance change with hysteresis as the polymer is oxidised or reduced.

For a negative control the polymers were first cycled in Rh control (Human, Bovine serum, Gamma Biologicals) before being cycled in Blood Grouping Reagent Anti-D (Gamma Biologicals), a monoclonal\polyclonal blend.

It is important to realise that cyclic resistometry measures the total resistance of the cell solution and the polymer. Before addition of anti-D the resistogram changes markedly as a function of the number of cycles. When antibody is added this will cause changes in solution resistance that will impact on the overall result. Thus, blank polymers not containing red cells show changes in resistance when antibody is added. This is a combination of solution resistance and non specific adsorption of antibody to the polymer.

Red blood cell containing polymers show different changes upon addition of antibody (anti-D) and antibody control (see Figure 14). However, when anti-D is added the change in resistance is inverted ie. the resistance change between 0.0V and -0.8V, it becomes negative instead of positive. These studies confirm that the cytopolymers may be useful in the detection and analysis of antibody-antigen interactions.

Using the strategy described herein it is possible to tailor the properties of the biomaterial in the following ways.

By changing the nature of the conducting polymer, eg. using substituted pyrroles or thiophenes, it is possible to change the redox properties (eg the potential at which the transition from oxidised to reduced state takes place) and also chemical properties (eg solubility of polymer).

By changing the polyani nic counterion it is possible to change the interaction of the final composite with different cellular elements in the body eg. use of heparin sulphate

provides a surface that resists the clotting of blood and adherence of platelets. Incorporation of other biologically active macromolecules such as collagens, fibronectins and specific adhesion molecules (integrins, selectins, cadherins etc) could cause selective interaction of certain desired cell types with the material. The latter material could be immobile in the polymer by design.

By changing the releasable component of the polymer selected pharmacological or physiological responses could be elicited. Illustrated here are four test cases (1) horseradish peroxidase, (2) haemoglobin, (3) human serum albumin and (4) nerve growth factor. Release of the latter protein causes profound changes in the behaviour of cells growing on the composite polymer. It causes them to stop dividing and to differentiate into cells containing long axonal processes. This test case illustrates the feasibility of release of any protein growth factor in a controlled fashion to cause cellular changes. It is envisaged that similar materials could be constructed containing for example endothelial cell growth factor that would induce arterial grafts to become lined with a full covering of endothelial cells *in vivo*. Such a result can only be performed in the present art by pre-coating arterial grafts *in vitro* prior to transplantation. The present art describes the controlled release of drugs having a low molecular weight (eg Dopamine, see Miller L. L. Zhou Q-X, Macromolecules 1987, 20, 1594) but not the release of proteins.

It can also be seen from the above examples that cytopolymers wherein the cell remains bioactive may be useful as biosensors or diagnostic tools. Such tools may be useful in, for example, various biological reactions such as the reaction of an antigen incorporated in the polymer with an antibody specific for that antigen.

Such structures have been found to have a high water content and to be hygroscopic. This environment is suited for cell integration and allows access of proteins (eg. analytes or antibodies) to the cell surface or receptors in subsequent sensor uses. By using different concentrations or a combination of counterions, it is possible to vary the number and state of the cells of the polymer.

Any viable cell may be used in accordance with the invention. Red blood cells are probably the most fragile of cells and thus provide a suitably rigorous test system. Other cells, such as white blood cells (granulocytes, monocytes/macrophages, lymphocytes), for detection of anti-HIV or gp^{140}, or for analysis of MHC antigens, stable cell lines or

primary cultures of liver, kidney or other tissue cells, for assessment of cell surface markers, receptors and their respective tumour cell lines, whether primary or stable cell lines, may be used. Which cell is incorporated depends upon the ultimate use required of the cytopolymer. Sucrose, dextrose, mannitol, trehalose and other agents known for their ability to aid in sustaining cell viability and integrity through providing an appropriate energy source or isotonicity may also be incorporated during the synthetic process. Clearly the extent to which cells will remain "viable" after polymerisation will be different for each cell type and will depend on what property of the cell (for example an antigen's ability to bind to antibody) is being utilised in the specific cytopolymer.

Although the foregoing describes specific embodiments of the invention, modifications apparent to the skilled addressee fall within the scope of the invention.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A conductive electroactive composite polymer comprising a hydrophilic counterion in combination with a biological macromolecule or and/a biological macromolecular complex.

2. A polymer according to claim 1, wherein the counterion is a polyelectrolyte.

3. A polymer according to claim 1 or claim 2, wherein the polyelectrolyte is biologically derived.

4. A polymer according to any one of claims 1 to 3, wherein the polyelectrolyte is a mucopolysaccharide.

5. A polymer according to any one of claims 1 to 3, wherein the polyelectrolyte is selected form the group consisting of polyvinyl sulphonate, dextran sulphate, chondroitin sulphate, polyglutamic acid, polyacrylic acid, heparin sulphate and hyaluronic acid.

6. A polymer according to any one of the preceding claims, wherein the macromolecule is a protein, a glycoprotein or a lipoprotein.

7. A polymer according to claim 6, wherein the protein is an enzyme, a hormone, a growth factor or an integral cell membrane protein.

8. A polymer according to any one of claims 1 to 5, wherein the macromolecular complex is a virus.

9. A polymer according to any one of claims 1 to 5, wherein the macromolecular complex is a bioactive cell.

10. A polymer according to claim 9, wherein the cell is selected form the group consisting of a bacterial cell, a fungal cell, a plant cell, or an animal cell.

11. A polymer according to claim 9 or claim 10, wherein the cell is a red blood cell.

12. A polymer according to any one of claims 1 to 5 or 9 to 11, wherein the polymer comprises a composite of polypyrrole:polyvinyl sulphonate in communication with a red blood cell.

13. A polymer according to any one of claims 1 to 8, wherein the biological macromolecule or macromolecular complex is releasable from the composite polymer.

14. A polymer according to any one of the preceding claims, wherein the polymer is biocompatible.

15. A process for making a conductive electroactive composite polymer having a hydrophilic counterion comprising the step of oxidising a monomer in the presence of a hydrophilic counterion and a biological macromolecule and/or a biological macromolecular complex.

16. A process according to claim 15, wherein the monomer is electro-oxidised.

17. A process according to claim 15, wherein the monomer is oxidised by chemical means.

18. A process according to any one of claims 15 to 17, wherein the monomer is capable of being polymerised into a conductive electroactive polymer.

19. A process according to claim 18, wherein the monomer is selected from the group consisting of pyrrole, aniline and thiophene.

20. A process according to any one of claims 15 to 19, wherein the counterion is a polyelectrolyte.

21. A process according to any one of claims 15 to 20, wherein the polyelectrolyte is of biological origin.

22. A process according to claim 21, wherein the derived polyelectrolyte is selected form the group consisting of polyvinyl sulphonate, dextran sulphate, chondroitin sulphate, polyglutamic acid, polyacrylic acid, heparin sulphate and hyaluronic acid.

23. A process according to any one of claims 15 to 22, wherein the macromolecule is a protein, a glycoprotein or a lipoprotein.

24. A process according to claim 23, wherein the protein is an enzyme, a hormone, a growth factor or an integral cell membrane protein.

25. A process according to any one of claims 15 to 22, wherein the macromolecular complex is a virus.

26. A process according to any one of claims 15 to 22, wherein the macromolecular complex is a bioactive cell

27. A process according to claim 26, wherein the cell is selected form the group consisting of a bacterial cell, a fungal cell, a plant cell or an animal cell.

28. A process according to any one of claims 15 to 25, wherein the biological macromolecule or macromolecular complex is releasable from the composite polymer.

29. A process according to any one of claims 15 to 28, wherein the polymer is biocompatible.

30. A process according to any one of claims 15 to 29, conducted at a temperature below 10°C.

31. A method of delivering a biological macromolecule contained within a conductive electroactive composite polymer into an environment surrounding the polymer, said polymer comprising a hydrophilic counterion in combination with a biological macromolecule and/or a biological macromolecular complex, said method comprising the step of:

applying a stimulus to said composite polymer to release said macromolecule into said surrounding environment from said composite polymer.

32. A method according to claim 31, wherein the stimulus is biochemical, chemical or electrochemical reduction or oxidation.

33. A method of detecting a ligand comprising the steps of:

a) introducing a conductive electroactive composite polymer comprising a hydrophilic counterion in combination with a biological macromolecule and/or a biological macromolecular complex into an environment containing a ligand which specifically binds to said macromolecule or macromolecular complex contained within the composite polymer;

b) allowing the ligand to bind to said macromolecule and/or macromolecular complex;

c) measuring the change in electrical properties of said composite polymer and thereby determining the presence and/or concentration of the ligand.

34. A method according to claim 33, wherein the ligand is an antibody.

35. A method according to claim 33, wherein the macromolecule is a protein.

36. A method according to claim 33, wherein the macromolecular complex is a virus.

37. A method according to claim 33, wherein the macromolecular complex is a bioactive cell.

38. A method according to claim 36, wherein the cell is selected form the group consisting f a bacterial cell, a fungal cell, a plant cell, or an animal cell.

39. A method according to claim 37 or claim 38, wherein the cell is a red blood cell.

40. A method according to any one of claims 33, 34 or 37 to 39, wherein the polymer comprises a composite of polypyrrole:polyvinyl sulphonate in communication with a red blood cell.

41. A method according to any one of claims 33 to 40, wherein the change in electrical property measured is a change in the resistance of the polymer.

42. A method according to any one of claims 31 to 41, wherein the environment is the body of an animal host.

43. A method according to any one of claims 31 to 42, wherein the environment is a fluid sample.

44. A method according to any one of claims 31 to 43, wherein the sample is a biological fluid sample.

45. A method of preventing or treating a disorder caused by or associated with a deficiency or absence of a biological macromolecule and/or a biological macromolecular complex comprising the step of administering to a host requiring such treatment a conductive electroactive polymer according to any one of claims 1 to 14.

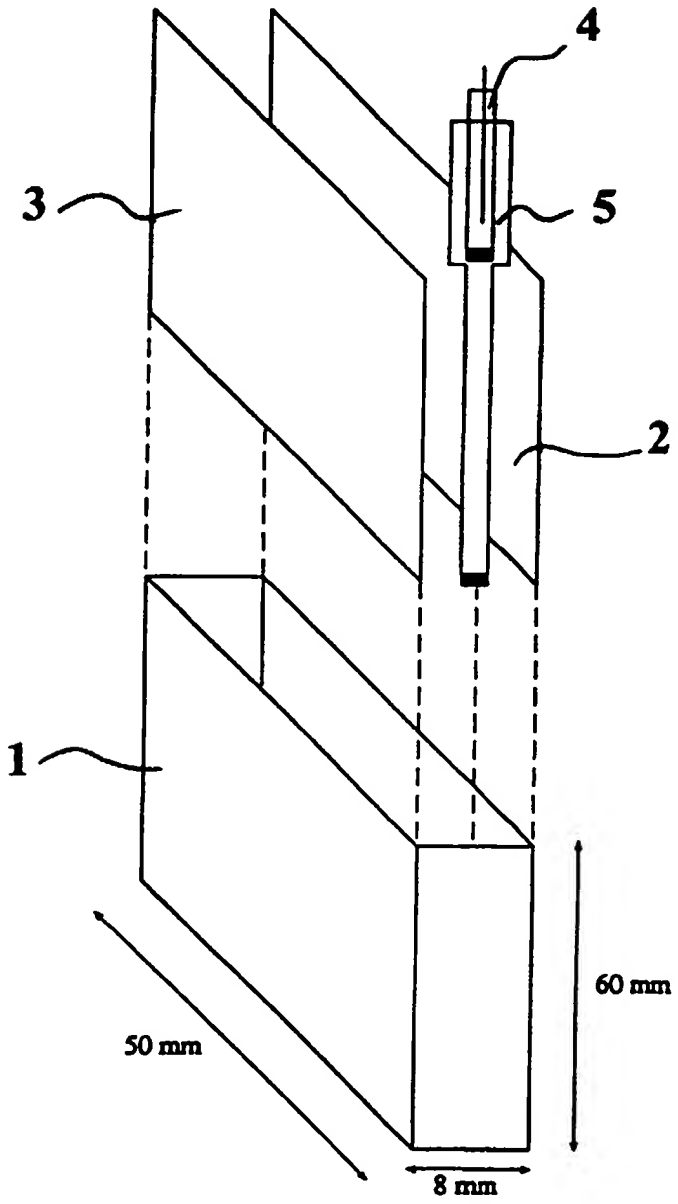


Fig. 1

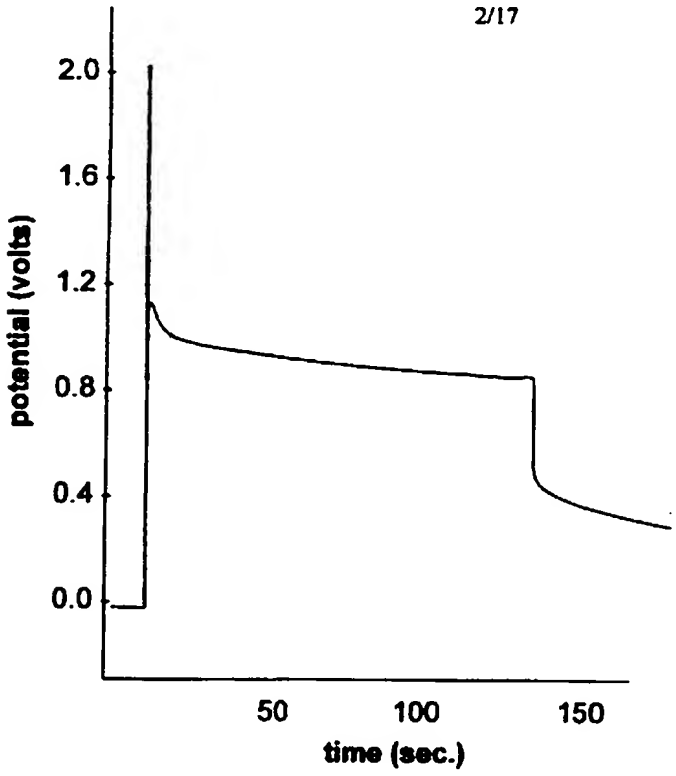


Fig. 2A

Fig. 2B

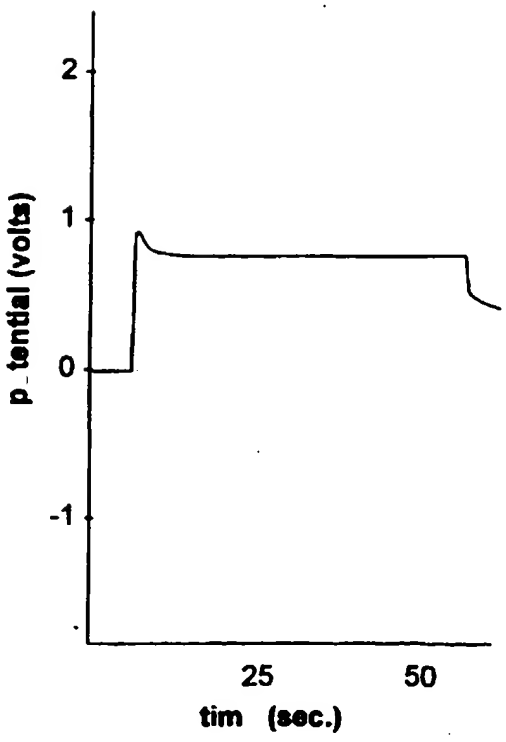
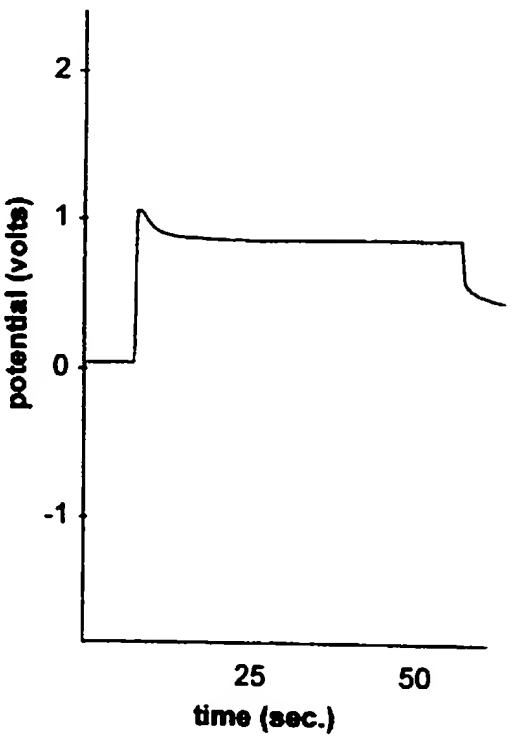
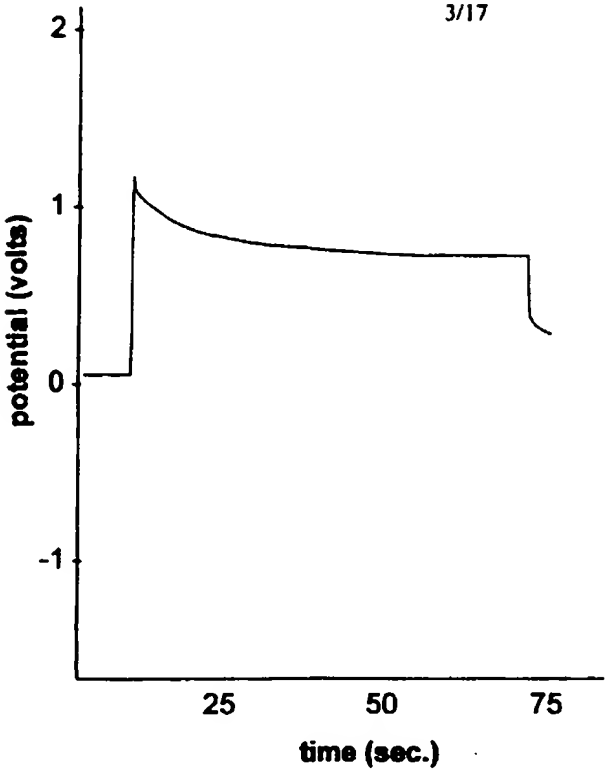


Fig. 2C

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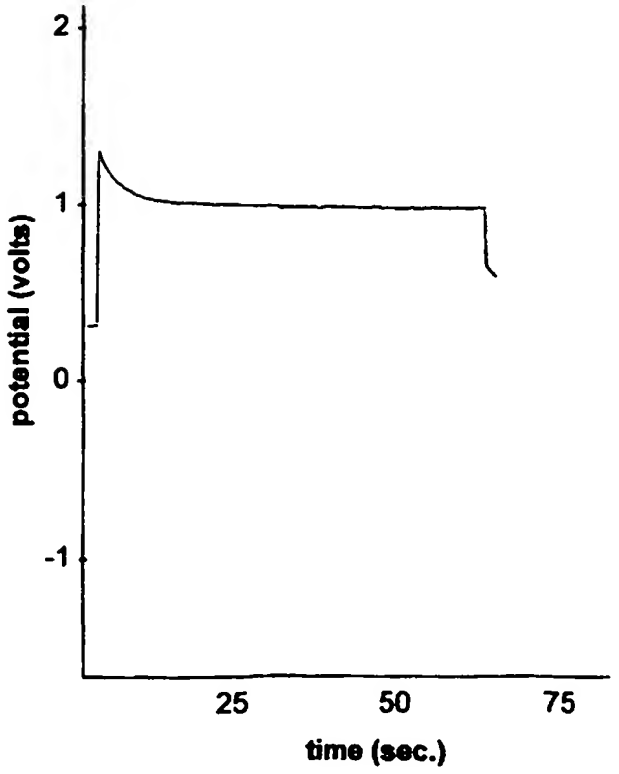
potential (volts)
25
50
75
time (sec.)

Fig. 2D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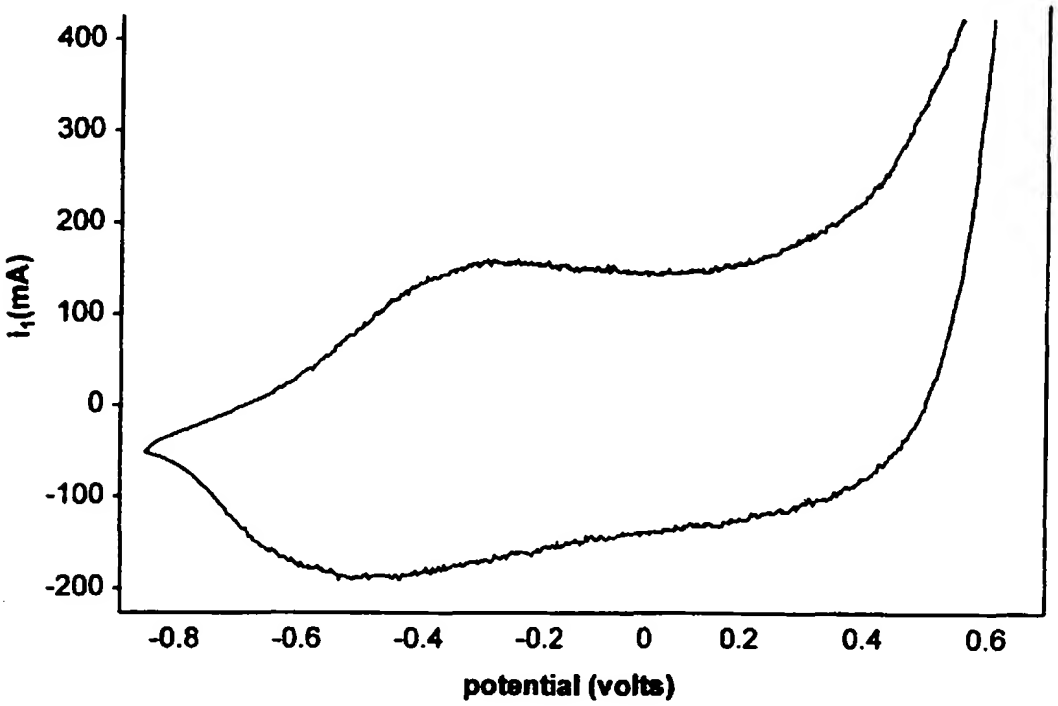
time (sec.)

Fig. 2E

Fig. 3A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300
200
100
0
-100
-200
I_1(mA)
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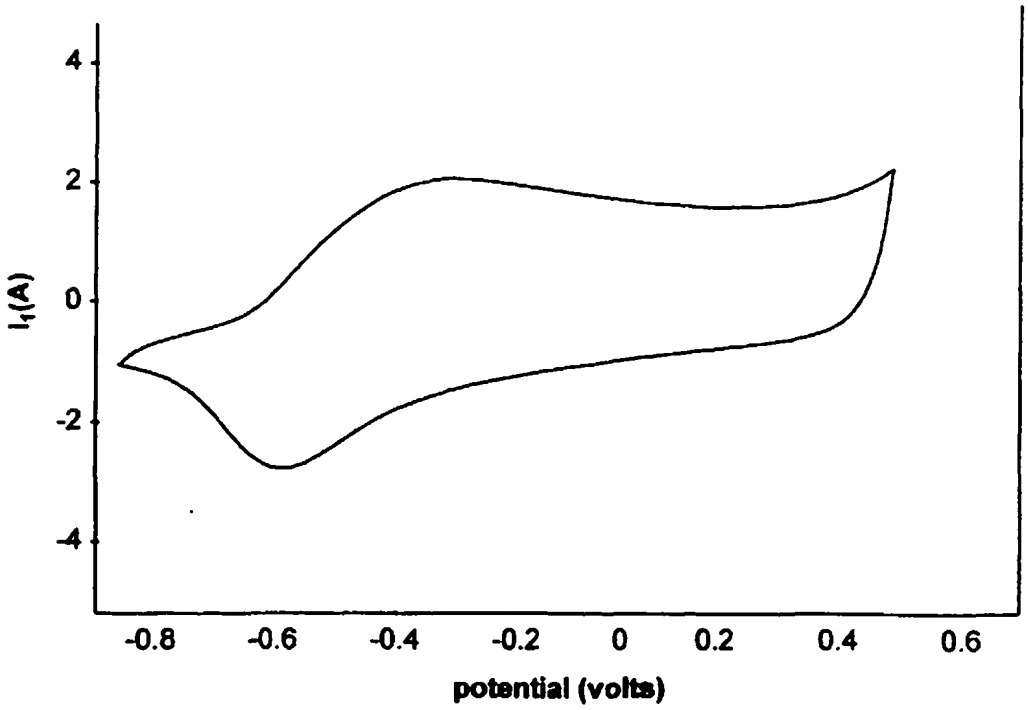
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
potential (volts)

Fig. 3B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I_1(A)
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0
0.2
0.4
0.6
potential (volts)

Fig. 3C

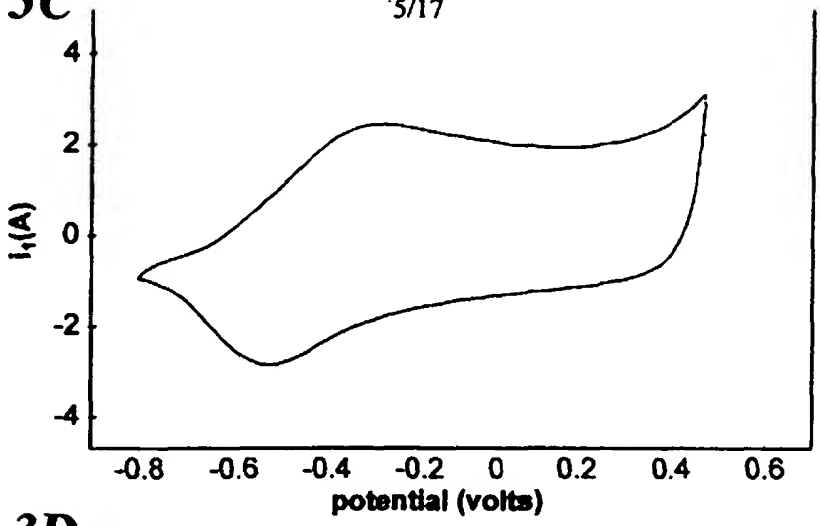


Fig. 3D

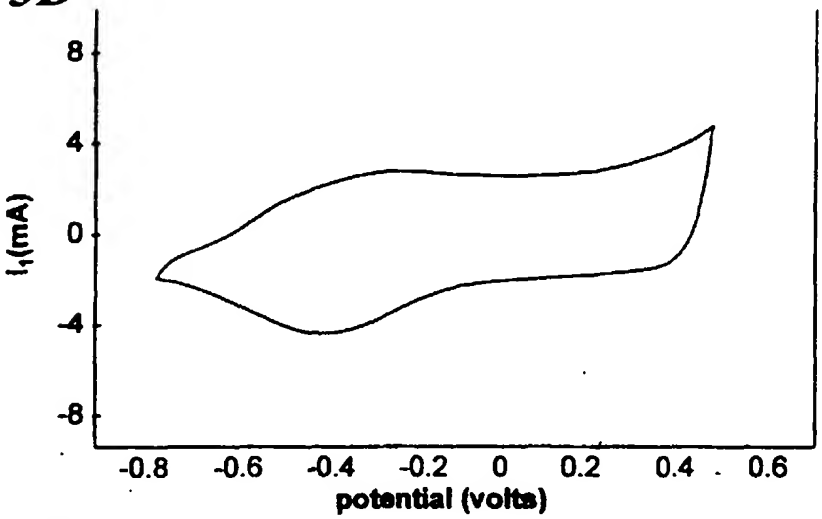


Fig. 3E

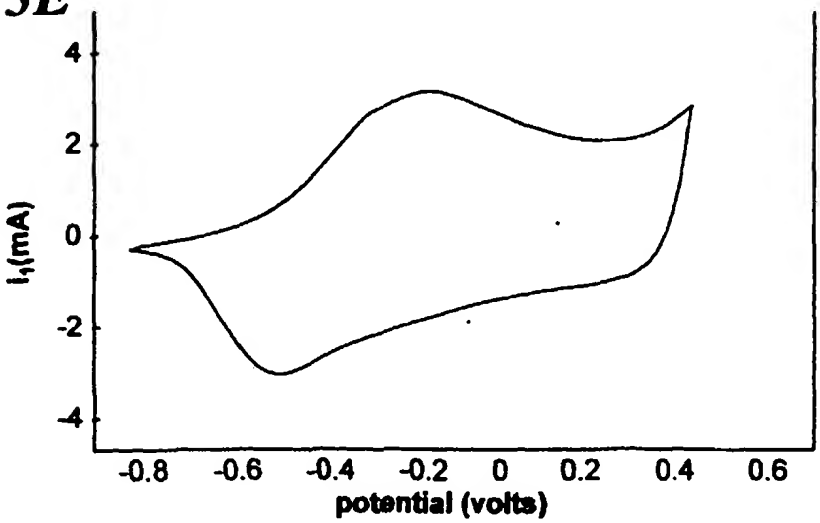


Fig 4A

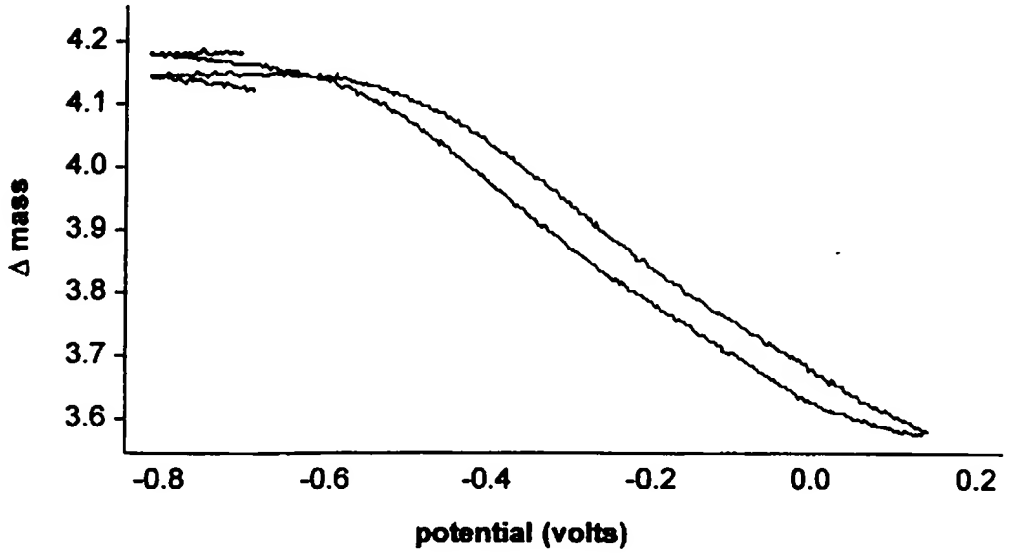
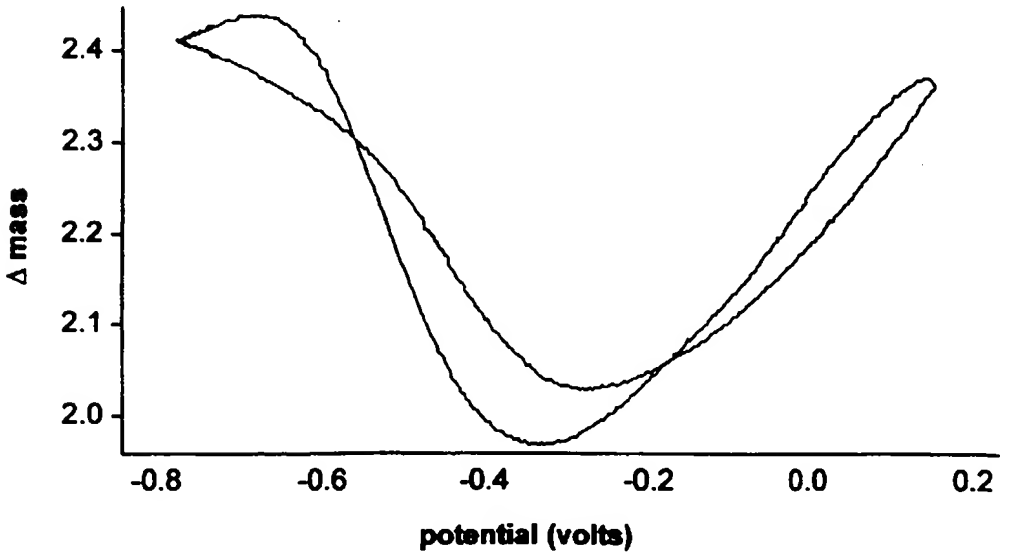

4.2
4.1
4.0
3.9
3.8
3.7
3.6
Δ mass
-0.8
-0.6
-0.4
-0.2
0.0
0.2
potential (volts)

Fig. 4B


2.4
2.3
2.2
2.1
2.0
Δ mass
-0.8
-0.6
-0.4
-0.2
0.0
0.2
potential (volts)

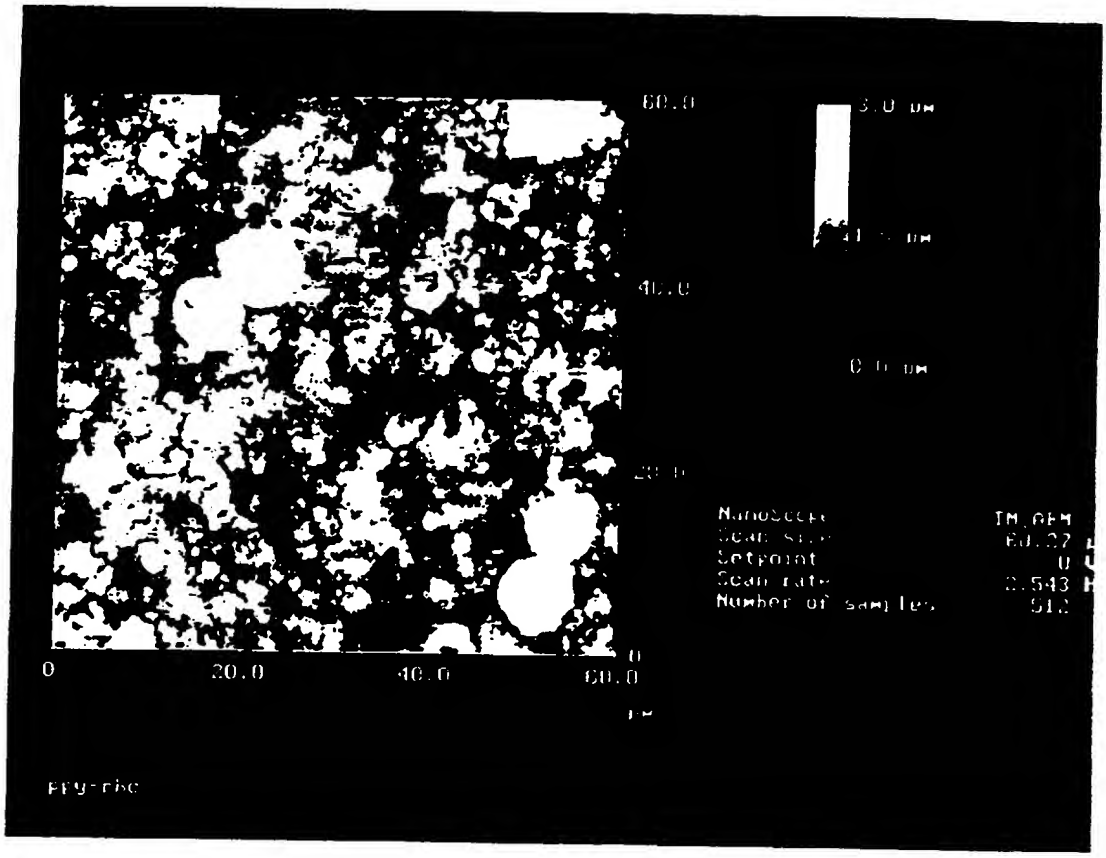

60.0
40.0
20.0
0
0
20.0
40.0
60.0
Setpoint
Scan rate
Number of samples
512
Pry-rbc

Fig. 5

Fig. 6B

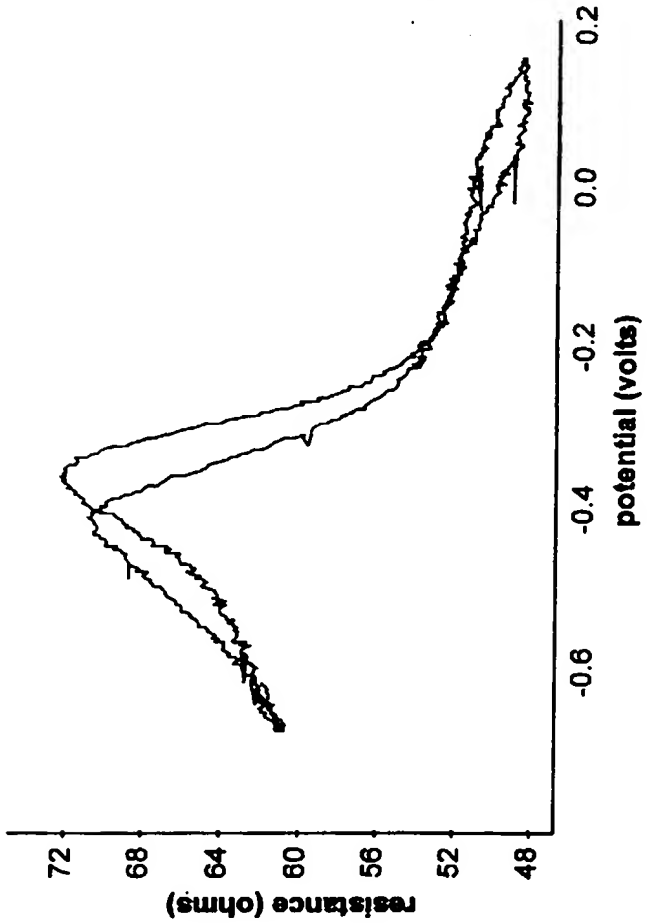
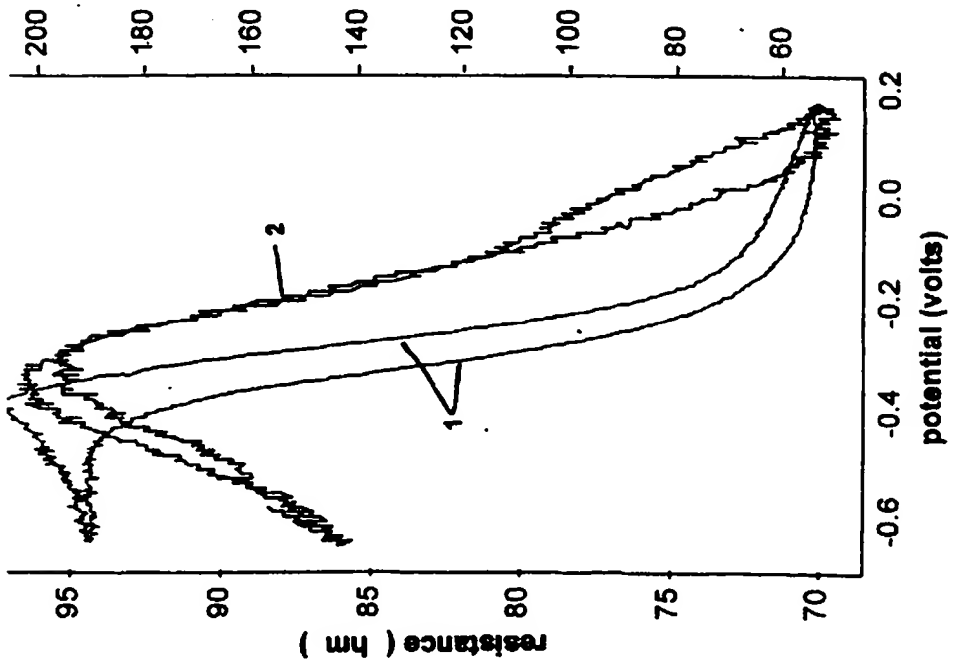

resistance (ohms)
72
68
64
60
56
52
48
potential (volts)
0.2
0.0
-0.2
-0.4
-0.6

Fig. 6A


resistance (hm)
95
90
85
80
75
70
200
180
160
140
120
100
80
60
potential (volts)
0.2
0.0
-0.2
-0.4
-0.6
1
2

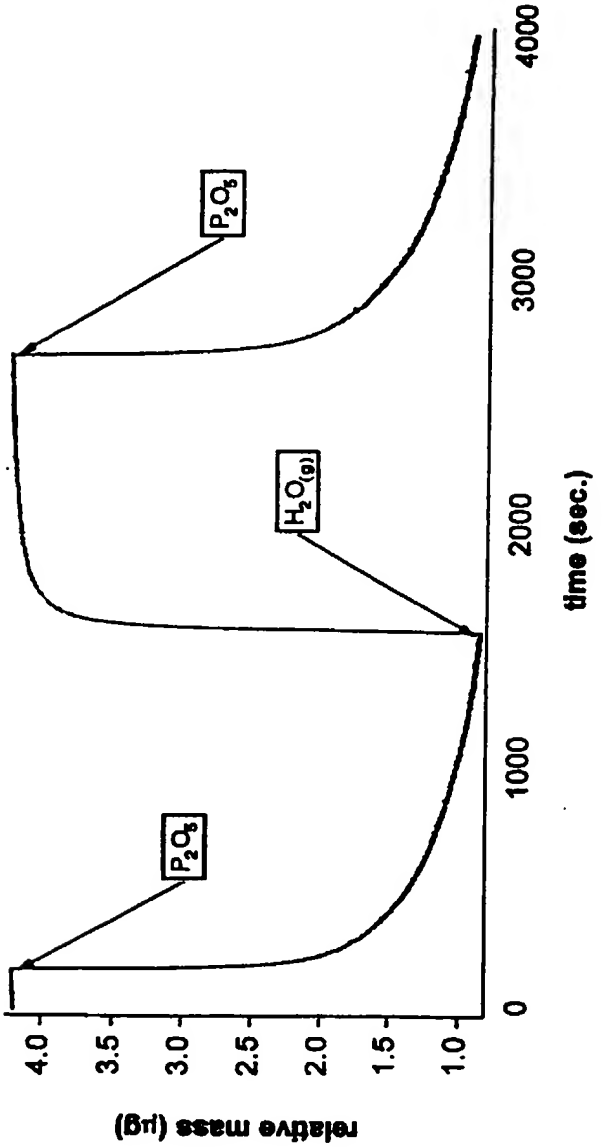

relative mass (µg)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0
1000
2000
3000
4000
time (sec.)
P_2O_5
$H_2O_{(g)}$
P_2O_5

Fig. 7

Fig. 8A

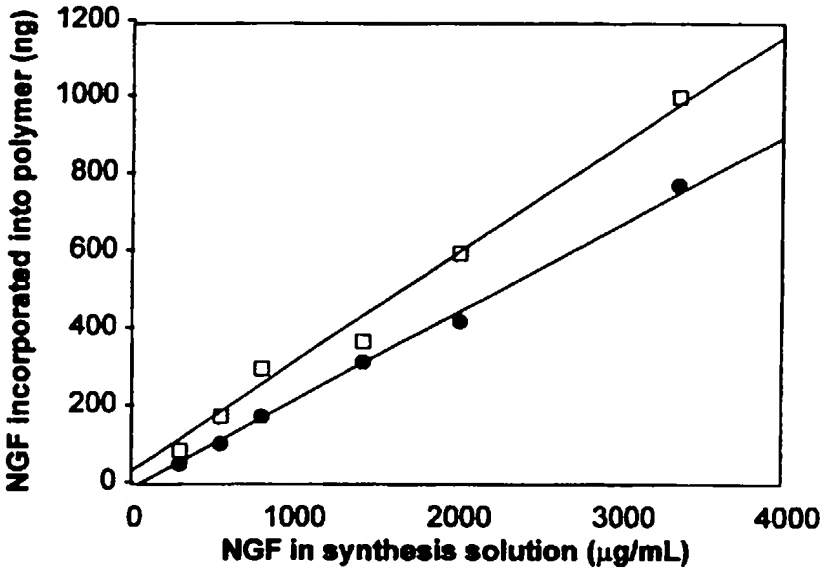
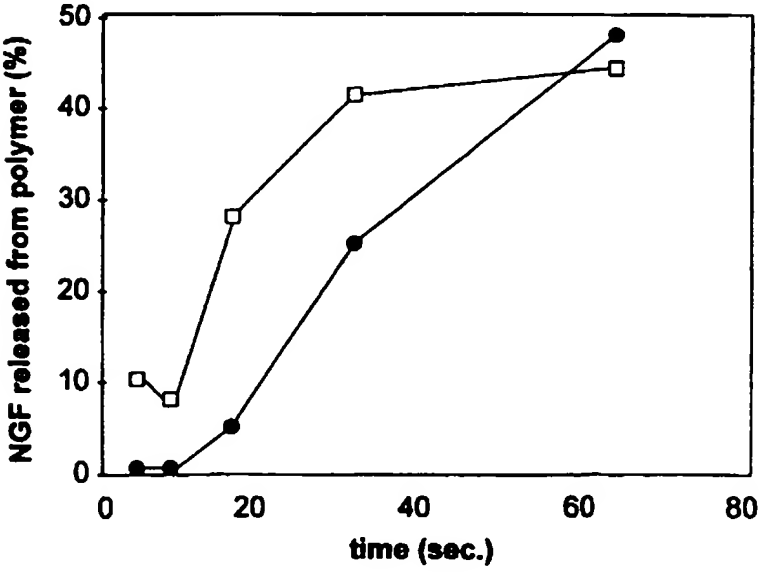

NGF incorporated into polymer (ng)
1200
1000
800
600
400
200
0
0
1000
2000
3000
4000
NGF in synthesis solution (µg/mL)

Fig. 8B


NGF released from polymer (%)
50
40
30
20
10
0
0
20
40
60
80
time (sec.)

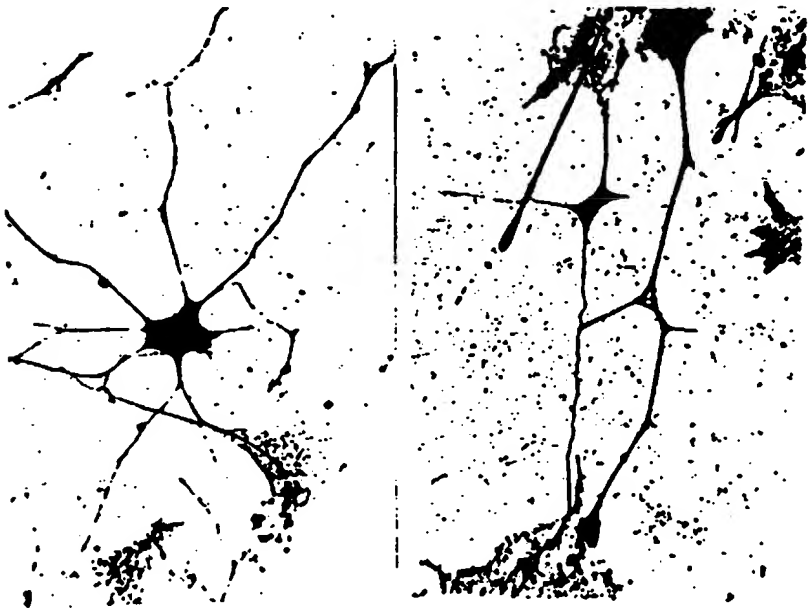


Fig. 9

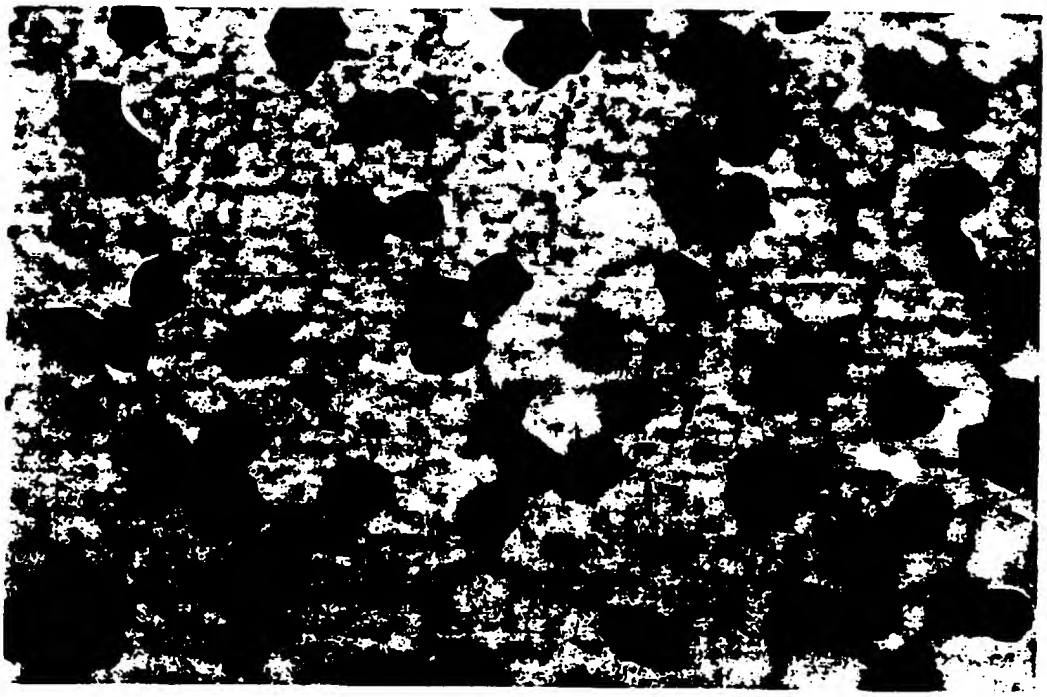


Fig. 10

Fig. 11

Fig. 12A

Fig. 12B

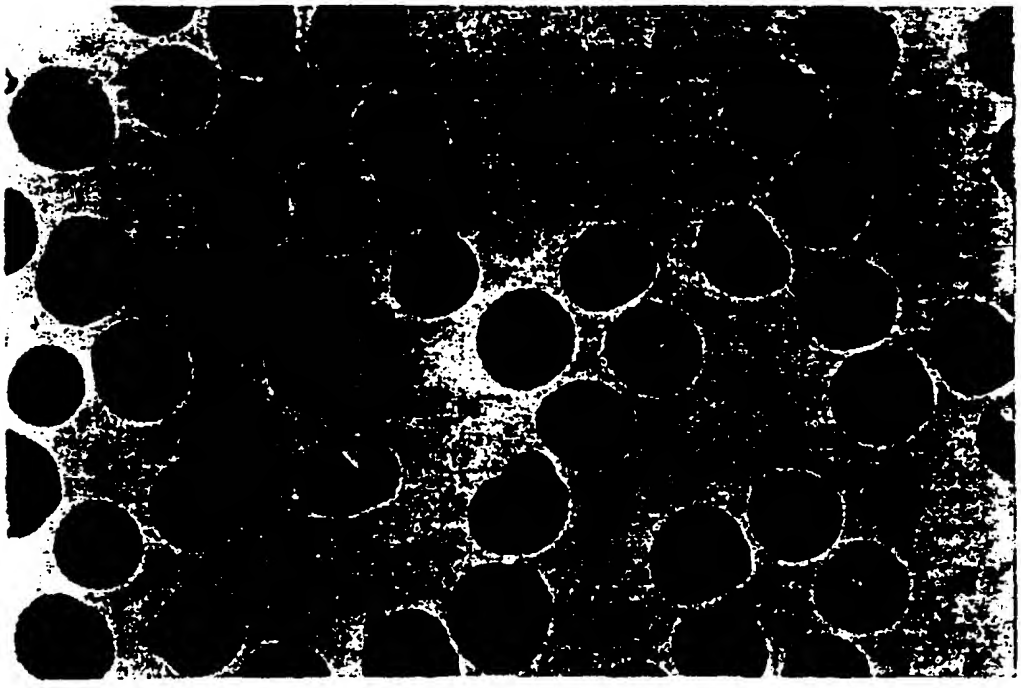


Fig. 13A

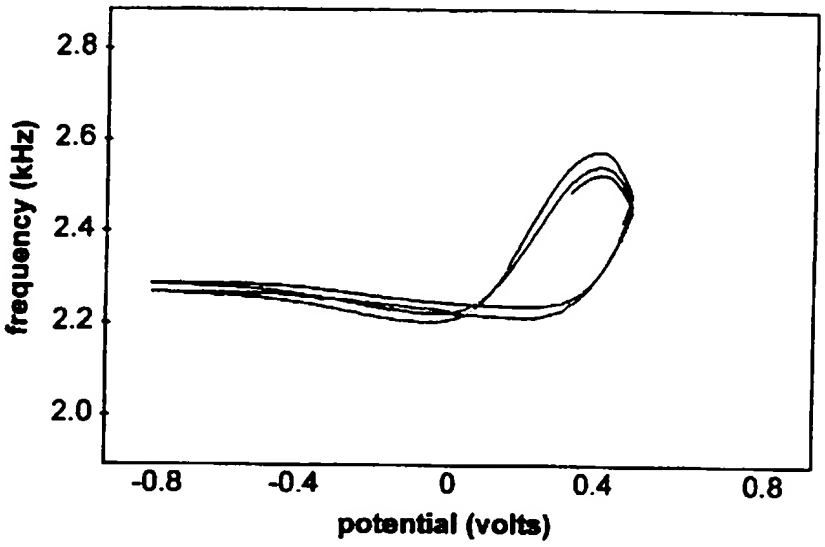


Fig. 13B

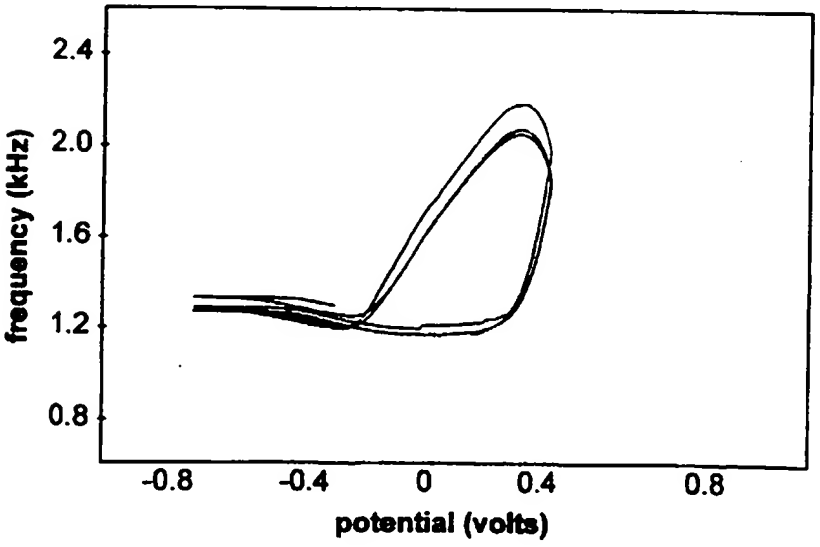


Fig. 13C

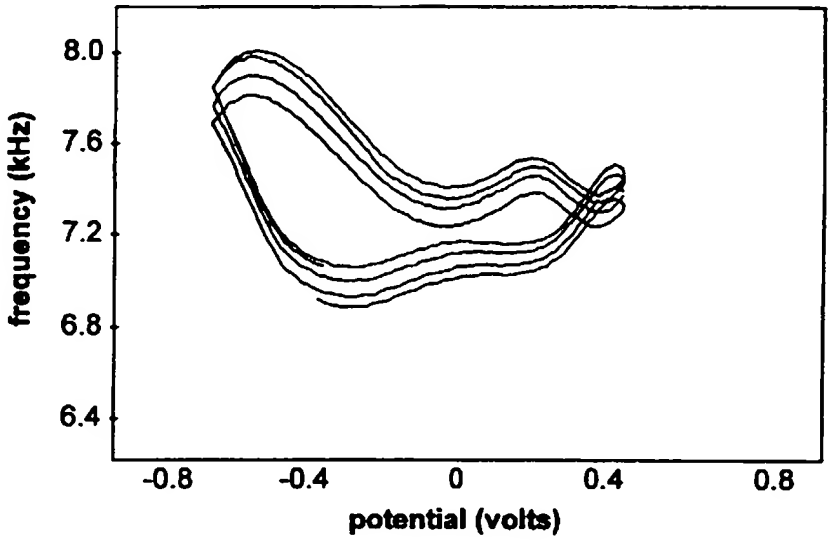


Fig. 13D

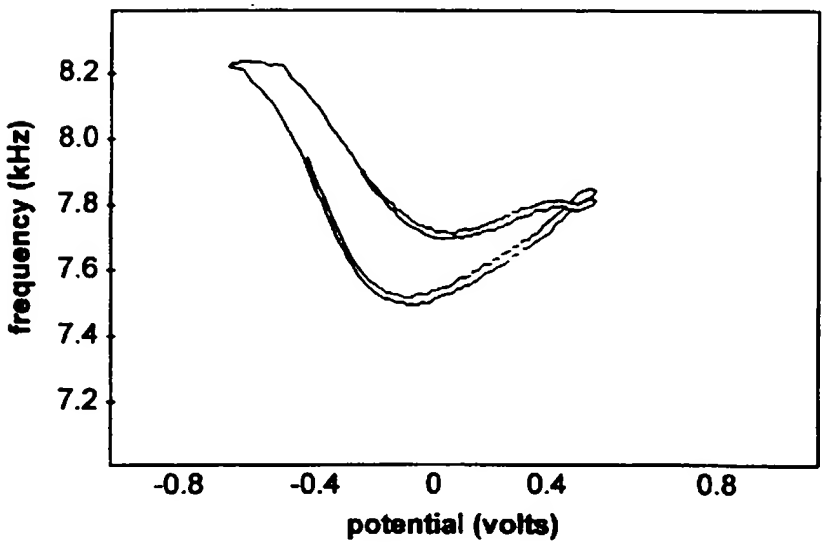


Fig. 14A

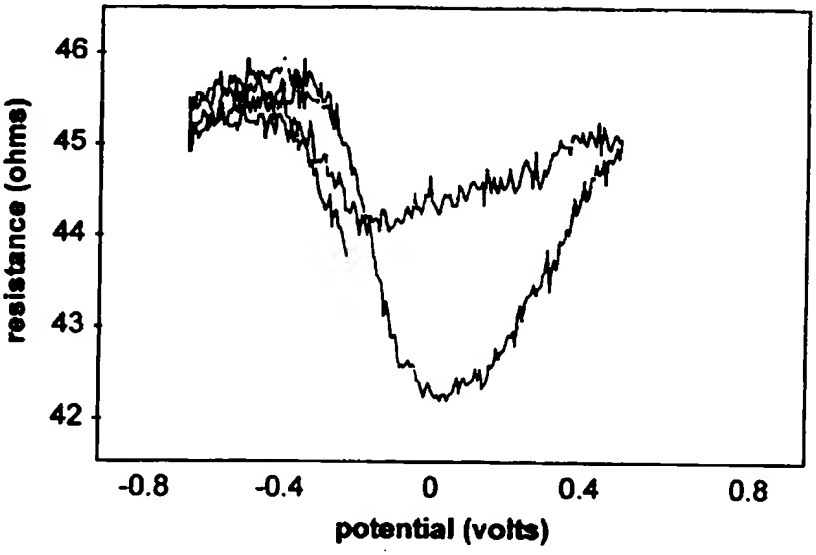
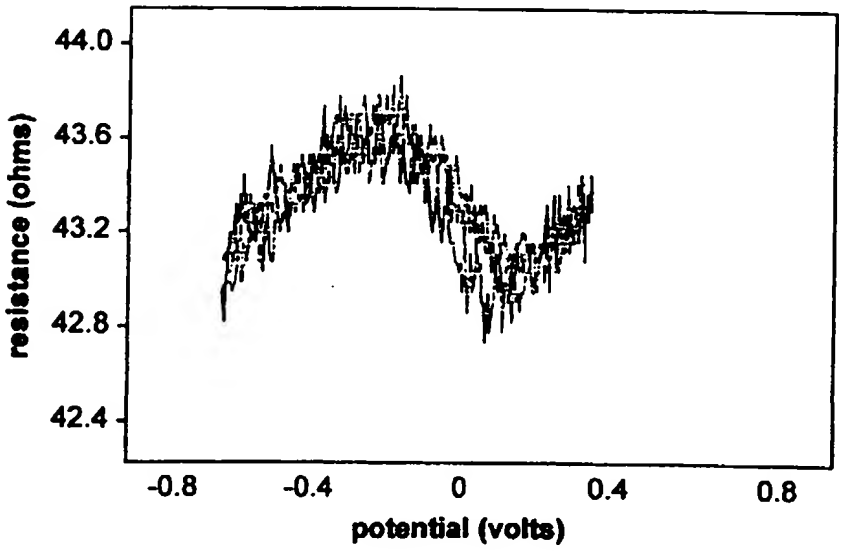


Fig. 14B



INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 95/00473

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C08L 99/00, 89/00, A61K 47/00, 47/32, 47/34, 47/48, C12N 11/04, 11/08, 11/10, C08H 1/00, G01N 33/566, C07K 17/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: C08L 99/00, 89/00, A61K 47/00, 47/32, 47/34, 47/48, C12N 11/04, 11/08, 11/10, C08H 1/00, G01N 33/566, C07K 17/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU:IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT: CONDUCT: ELECTR:
JAPIO: CONDUCT: ELECTR:

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU, A, 91652/91 (AKERMES CONTROLLED THERAPEUTICS INC.) 17 AUGUST 1992 page 4 - page 11; Claims 1-24	1-3, 6, 9-10, 13-14, 45
X	AU, A, 81081/91 (ALBANY MEDICAL COLLEGE) 10 DECEMBER 1991 pages 1; 5; 7; 9; 11; Claims 1-40	1-10, 13-14, 33, 45
X	AU, A, 90986/82 (SOCIETE ANONYME DE DEVELOPMENT DE UTILISATIONS DU CUR) 9 June 1983 pages 2;4;6; claims 1 to 8	1-5; 45

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

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Date of the actual completion of the international search	Date of mailing of the international search report
14 November 1995	16 NOVEMBER 1995

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 95/00473

C (Continuation) **DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
X	GB, A, 2080814 (CESKOSLOVENSKA AKADEMIE VED PRAHA CZECHOSLOVAKIA) 10 FEBRUARY 1982 pages 1; 2; Claims 1 to 9	1-3; 45
A	AU, A, 34454/89 (MILLS, Randell) 3 NOVEMBER 1989 page 1; Claims 1 to 40	1-45
A	AU, B, 70829/87 (604687) (LA JOLLA PHARMACEUTICAL COMPANY) 22 DECEMBER 1987 pages 1; 5-6; Claims 1 to 9	1-45
A	AU, A, 50445/85 (BEECHAM GROUP P.L.C.) 17 JULY 1986 pages 1; 2; 15; Claims 1 to 15	1-45
A	AU, B, 84714/75 (495261) (PHARMACEUTICAL SOCIETY OF VICTORIA AND PETER SPEISER) 17 MARCH 1977 pages 1; 4; 12; Claims 1 TO 18	1-45
A	EP, A, 46136 (CIBA-GEIGY AG) 17 FEBRUARY 1982 pages 4; 23-26; Claims 1 to 25	1-45
A	US, A, 4585652 (MILLER, et al) 29 APRIL 1986 columns 2; 4; 6; Claims 1 to 19	1-45

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/AU 95/00473

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AU	91652/92	CA	2099376	EP	565618	JP	7503700
		WO	9211844	JP	4504685	DE	4003801
		EP	466890	FI	914720	US	5271302
		WO	9112118				
AU	81081/91	EP	607126	US	5216130	WO	9118020
		WO	9513445	FI	934959	FI	94659
AU	90986/82	CA	1175353	DE	3272938	EP	81440
		FR	2517315	JP	58103322	US	4451397
		AT	129536	BR	9107186	EP	563150
		EP	665329	FI	932608	FI	951522
		JP	6503385	NO	932162	SE	9004009
		SE	468602	US	5360656	WO	9211411
GB	2080814	CS	216992	DE	3128815	FR	2493328
		US	4563490	JP	57052460	JP	61022586
		US	4427808				
AU	34454/89	EP	414730	JP	3505574	WO	8909833
		CN	1047075	WO	9316254		
AU	70829/87	AT	90876	CA	1309558	DE	3786334
		DK	334/88	EP	272278	JP	63503457
		NO	880376	WO	8707145	US	4950713
		US	4950469	US	5017648	NO	910541
		BR	9100564	EP	446976	EP	662770
		US	5069280	WO	9501704		

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International Application No.
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		JP	6279318	JP	7017517	PT	81581
		WO	9009303	US	4935465	EP	429559
		GB	8902640	GB	9002483	GB	2228456
		JP	3503753	US	5094503		
AU	84714/75	GB	1516348	US	4107288	BR	8203393
		DE	3123034	EP	66802	IN	156556
		JP	57211974	ZA	8204049	CA	2055381
		EP	479093	JP	4264093	JP	6102673
		NZ	239974	US	5298616	ZA	9107707
EP	46136	DE	3163877	US	4423099	JP	57055967
		JP	3010667				
US	4585652	AT	79745	CA	1266887	DE	3586560
		EP	182765	JP	61196967	JP	3045661
		US	4585652				

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